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Derivation of Human Induced Pluripotent Stem Cells from Patients with Maturity Onset Diabetes of the Young*[S]

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Background: Human induced pluripotent stem cells (hiPSCs) can be harnessed for development of novel therapeutics for metabolic disorders.
Results: Karyotypically normal hiPSCs were derived from patients with MODY1, MODY2, MODY3, MODY5, or MODY8.
Conclusion: hiPSCs were successfully derived from a variety of MODY patients.
Significance: MODY-hiPSCs can be used to explore the role of MODY genes in the development and function of pancreatic islet cells.

Maturity onset diabetes of the young (MODY) is an autosomal dominant disease. Despite extensive research, the mechanism by which a mutant MODY gene results in monogenic diabetes is not yet clear due to the inaccessibility of patient samples. Induced pluripotency and directed differentiation toward the pancreatic lineage are now viable and attractive methods to uncover the molecular mechanisms underlying MODY. Here we report, for the first time, the derivation of human induced pluripotent stem cells (hiPSCs) from patients with five types of MODY: MODY1 (*HNF4A*), MODY2 (*GCK*), MODY3 (*HNF1A*), MODY5 (*HNF1B*), and MODY8 (*CEL*) with a polycistronic lentiviral vector expressing a Cre-excisable human "stem cell cassette" containing the four reprogramming factors *OCT4*, *KLF4*, *SOX2*, and *CMYC*. These MODY-hiPSCs morphologically resemble human pluripotent stem cells (hPSCs), express pluripotency markers OCT4, SOX2, NANOG, SSEA-4, and TRA-1–60, give rise to derivatives of the three germ layers in a teratoma assay, and are karyotypically normal. Overall, our MODY-hiPSCs serve as invaluable tools to dissect the role of MODY genes in the development of pancreas and islet cells and to evaluate their significance in regulating beta cell function. This knowledge will aid future attempts aimed at deriving functional mature beta cells from hPSCs.

Maturity onset diabetes of the young (MODY)[2] is an autosomal dominant monogenic diabetic disease typically affecting individuals before the age of 25 years. To date, 11 MODY genes have been identified, comprising both transcription factors and enzymes/hormones: MODY1 (*HNF4A*), MODY2 (*GCK*), MODY3 (*HNF1A*), MODY4 (*PDX1*), MODY5 (*HNF1B*), MODY6 (*NEUROD1*), MODY7 (*KLF11*), MODY8 (*CEL*), MODY9 (*PAX4*), MODY10 (*INS*), and MODY11 (*BLK*) (1). Various disease-causing genetic variants have been identified for each of the MODY genes, all of which affect pancreas and beta cell development, ultimately resulting in beta cell dysfunction and diabetes.

The advent of induced pluripotency (2) has provided an opportunity to derive human induced pluripotent stem cells (hiPSCs) from MODY patients to model the disease *in vitro*. Several studies have reported that human somatic cells, commonly skin fibroblasts, can be reprogrammed into hiPSCs. A number of groups have reported the generation of hiPSCs from patients with mitochondrial diabetes, type 1 and type 2 diabetes mellitus (3, 4). However, given the complexity of type 1 and type 2 diabetes mellitus, the use of hiPSCs from these patients requires comparison with multiple controls that are appropriate for the genetic and environmental context.[3] In the case of MODY-hiPSCs, however, the single mutation in each MODY gene would be primarily responsible for disease because the patients develop diabetes without an immune attack or invoking insulin resistance.

Here we demonstrate, for the first time, the ability to derive hiPSCs from patients with five different MODY conditions (MODY1, MODY2, MODY3, MODY5, and MODY8). The MODY-hiPSCs are morphologically, molecularly, and functionally indistinguishable from human embryonic stem cells (hESCs) and other reported hiPSCs, collectively known as human pluripotent stem cells (hPSCs). The availability of MODY-hiPSCs for *in vitro* disease modeling will serve the scientific community in two timely areas of research. First, the differentiation of MODY-hiPSCs along the pancreatic lineage presents a unique model to explore whether specific MODY genes and/or transcription factors play key roles during human pancreas development. This is especially significant considering that the efforts to derive functional mature beta cells from hPSCs have been hampered by a lack of complete understanding of human pancreas developmental biology, particularly of

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[2] The abbreviations used are: MODY, maturity onset diabetes of the young; hiPSC, human induced pluripotent stem cell; hESC, human embryonic stem cell; hPSC, human pluripotent stem cell; GCK, glucokinase; HNF, hepatocyte nuclear factor; CEL, carboxyl ester lipase.
[3] A. K. K. Teo, A. J. Wagers, and R. N. Kulkarni, submitted for publication.

the late developmental stages (5). Second, because the mechanisms that underlie defects in islet function in MODY patients are still not fully understood, the MODY-hiPSCs will serve as an invaluable resource for investigating the direct role of the transcription factors and genes involved in the regulation of beta cell secretory function.

EXPERIMENTAL PROCEDURES

Patient Selection, Skin Biopsies, and Cell Culture—Skin biopsies were obtained after informed consent from healthy controls and MODY patients at Joslin Diabetes Center and Haukeland University Hospital. All studies using human material were reviewed and approved by the Institutional Review Boards and in accordance with the principles of the Declaration of Helsinki. Four- to six-mm skin biopsies were obtained from the anterior aspect of the upper forearm and cultured in a 6-well plate.

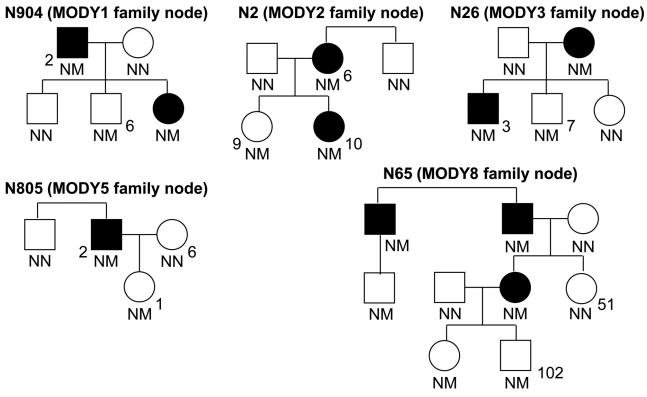
Sequencing of MODY Genes—Genomic DNA was harvested from patient skin fibroblasts and MODY-hiPSCs. MODY mutations were confirmed via PCR of known mutated regions of the MODY genes followed by Sanger sequencing.

Derivation and Characterization of hiPSCs—Derivation of some hiPSCs was performed in the Joslin iPS Core Facility. Further details regarding cell culture, primer sequences, generation of hiPSCs from patient skin fibroblasts (6), quantitative real time PCR, immunostaining, teratoma assay, and cytogenetic analysis can be found in the supplemental Experimental Procedures.

RESULTS

Characteristics of healthy individuals and MODY patients recruited at Joslin Diabetes Center and Haukeland University Hospital following informed consent are included in Fig. 1, *A* and *B*. We devised a family node design of 2–4 closely related subjects (a duo, trio, or quad design) to obtain appropriate controls for the MODY patients (further explained in the supplemental Experimental Procedures). Fig. 1*A* includes examples of family node pedigrees for MODY1, MODY2, MODY3, MODY5, and MODY8 families. Genomic DNA was harvested from cultured skin fibroblasts and MODY gene mutations (*HNF4A* (p.Ile271fs), *GCK* (V62A), *HNF1A* (P291fsinsC), *HNF1B* (S148L and g.1–1671del), and *CEL* (C563fsX673)) were independently confirmed via PCR followed by Sanger sequencing (Fig. 2, *A–E*, and data not shown).

We transduced the skin fibroblasts with human stem cell cassette (STEMCCA) Cre-excisable constitutive polycistronic (*OCT4*, *KLF4*, *SOX2*, and *CMYC*) lentiviruses and derived independent hiPSC clones from five healthy individuals (AG16102, 120111, 0922, N805-6, and N65-51) and 12 patients spanning five different MODYs (MODY1 (*HNF4A*), MODY2 (*GCK*), MODY3 (*HNF1A*), MODY5 (*HNF1B*), and MODY8 (*CEL*)) (Figs. 1*B* and 2F). Two of the control hiPSC lines were derived from healthy members of MODY5 (N805-6) and MODY8 (N65-51) families (Figs. 1, *A* and *B*, and 2*F*). MODY gene mutations were independently confirmed in the derived hiPSCs (supplemental Fig. S1, *A–E*). In general, skin fibroblasts from different individuals displayed variable reprogramming efficiencies (supplemental Fig. S1*F*).



B

Healthy controls			ID			Age (yrs)	Gender (M/F)	Source of fibroblasts
			AG16102			69	M	Coriell
			120111			84	M	Joslin
			0922			52	F	Joslin
MODY Patients	**Gene**	**Disease variant**	**ID**	**Mutation (Y/N)**	**Diabetes (Y/N)**	**Age (yrs)**	**Gender (M/F)**	**Source of fibroblasts**
1	*HNF4A*	p.Ile271fs	N904-2	Y	Y	51	M	Norway
			N904-6	Y	N	26	M	Norway
1	*HNF4A*	Unknown	GM1237	Unknown	Y	29	M	Coriell
2	*GCK*	V62A	N2-6	Y	Y	47	F	Norway
			N2-9	Y	N	23	F	Norway
			N2-10	Y	Y	20	F	Norway
3	*HNF1A*	P291fsinsC	N26-3	Y	Y	26	M	Norway
			N26-7	Y	N	18	M	Norway
5	*HNF1B*	S148L	N805-1	Y	N	11	F	Norway
			N805-2	Y	Y	43	M	Norway
			N805-6	N	N	41	F	Norway
5	*HNF1B*	g.1-1671del	N919-2	Y	Y	34	M	Norway
8	*CEL*	C563fsX673	N65-51	N	N	43	F	Norway
			N65-102	Y	N	16	M	Norway

FIGURE 1. **Summary of individuals recruited for this study.** *A*, family node pedigrees for MODY1, MODY2, MODY3, MODY5, and MODY8 families. *Squares* denote males, *circles* denote females, *solid symbols* denote diabetes, *NN* denotes no mutation and *NM* denotes mutation. *B*, clinical characteristics of individuals in the study. Individuals were de-identified and assigned an alternate identification number (*ID*). Age (years old) at which skin biopsies and skin fibroblasts were obtained, gender (male or female), and source of fibroblasts are indicated. Type of MODY condition (MODY1, MODY2, MODY3, MODY5, or MODY8), specific disease variant in the MODY gene, presence or absence of mutation (yes or no), and presence or absence of diabetes (yes or no) are indicated.

All hiPSCs derived in this study resemble hPSCs by morphologically presenting as distinct flat colonies (Fig. 2*F*), expressing pluripotency markers OCT4, SOX2, NANOG, SSEA-4, and TRA-1–60 (Fig. 2, *G* and *H*, supplemental Fig. S2, and data not shown), and exhibiting the ability to give rise to derivatives of the three germ layers (ectoderm, mesoderm, and definitive endoderm) in a teratoma assay (Fig. 2*I*, supplemental Fig. S1*G*, and data not shown). Interestingly, all the hiPSCs generated to date exhibit only one copy of viral integrant, allowing for easy excision when desired (supplemental Fig. S1*H*) (7). Normal karyotyping of MODY5-hiPSCs suggests that our method of reprogramming does not induce karyotypic abnormalities (Fig. 2*J* and data not shown).

DISCUSSION

We have successfully derived hiPSCs from healthy individuals and patients diagnosed with MODY1 (*HNF4A*), MODY2 (*GCK*), MODY3 (*HNF1A*), MODY5 (*HNF1B*), or MODY8 (*CEL*). These hiPSCs are morphologically, molecularly, and

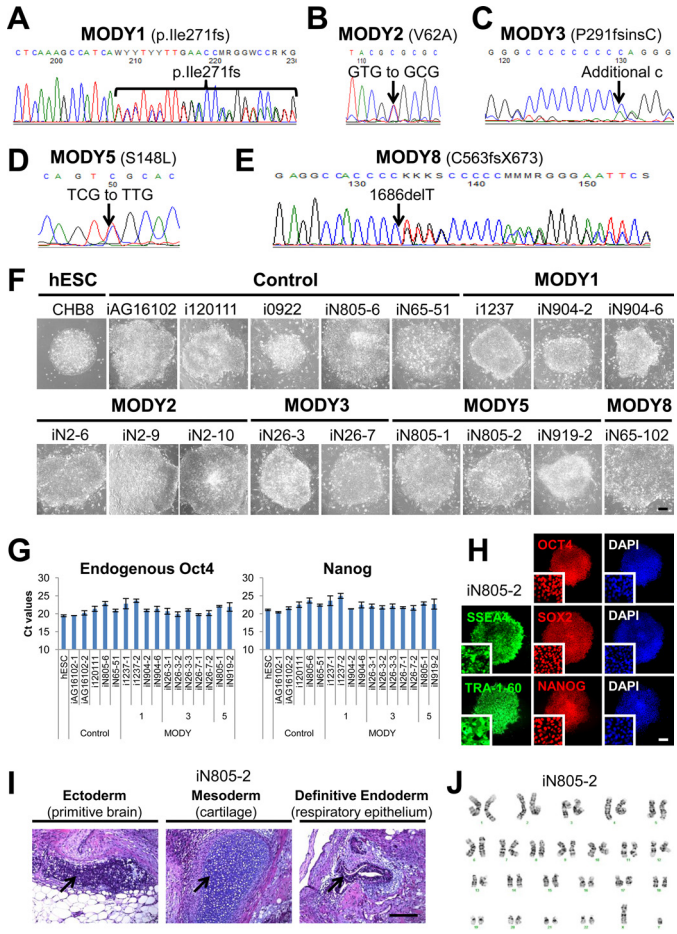


FIGURE 2. **Derivation of hiPSCs from healthy individuals and MODY patients.** *A–E*, MODY gene mutations were confirmed in MODY1 (p.Ile271fs) (N904-2 and N904-6) (*A*), MODY2 (V62A) (N2-6, N2-9, and N2-10) (*B*), MODY3 (P291fsinsC) (N26-3 and N26-7) (*C*), MODY5 (S148L) (N805-1 and N805-2) (*D*), and MODY8 (C563fsX673) (N65-102) (*E*) patient fibroblasts. *F*, the hiPSCs derived in this study morphologically resemble hESCs (CHB8, Daley laboratory). *Scale bar*: 200 μm. *G*, hiPSCs express endogenous *OCT4* and *NANOG* transcripts at comparable (high) levels to a control hESC line (CHB8). *H*, immunostaining analyses for OCT4, SOX2, NANOG, SSEA-4, and TRA-1–60 in a representative hiPSC line (MODY5; iN805-2). *Scale bar*: 200 μm. *I*, hiPSCs give rise to derivatives of the three germ layers: ectoderm (primitive brain), mesoderm (cartilage), and definitive endoderm (respiratory epithelium) in an *in vivo* teratoma assay. *Scale bar*: 200 μm. *J*, hiPSCs exhibit a normal karyotype in a G-band analysis.

functionally indistinguishable from hPSCs, and representative karyotyping of MODY5-hiPSCs indicates that our reprogramming approach does not induce karyotypic abnormalities. The use of a polycistronic lentiviral vector for reprogramming facilitates high reprogramming efficiencies yet, unlike the commonly used set of four independent retroviruses, does not result in a large number of integration sites. Because our hiPSCs were generated with floxed alleles, the single copy of integrated "stem cell cassette" can be excised using Cre recombinase to obtain integrant-free hiPSCs and avoid nonspecific effects. Together, these data indicate that our hiPSCs are unique tools for *in vitro* comparative disease modeling of the various MODY disorders.

The MODY-hiPSCs that we have derived are unique tools for *in vitro* disease modeling to study the role of *HNF4A*, *GCK*, *HNF1A*, *HNF1B*, and *CEL* in human pancreas and beta cell development, as well as in beta cell dysfunction. Using MODY-hiPSCs from nondiabetic mutation carriers further offers the potential advantage of studying prediabetic cell biology. *HNF4A* (MODY1), *HNF1A* (MODY3), and *HNF1B* (MODY5) are members of the hepatocyte nuclear factor (HNF) family, implicated in pancreas development and function (8). HNF4A is important for the development and function of both beta cells and hepatocytes. Because MODY1 patients exhibit a diabetic phenotype that has been attributed to defective glucose-stimulated insulin secretion, the availability of MODY1-hiPSCs will allow *in vitro* modeling to determine the relevance of *HNF4A* mutations for liver development *versus* beta cell function. The transcriptional regulation of *HNF1A* by HNF4A (8) could partially account for the phenotypic similarities between the beta cells of MODY3 and MODY1 patients. As both MODY1 and MODY3 patients frequently exhibit diabetic microvascular complications, their hiPSCs can be used to derive endothelial cells to investigate mechanisms contributing to the complications. HNF1B has a critical role in both pancreas and kidney development. MODY5 patients are known to develop pancreatic hypoplasia and/or agenesis, and several patients manifest hypoplastic glomerulocystic kidney disease that is independent of the diabetes. Thus, MODY5-hiPSCs can be used to investigate the impact of mutations in *HNF1B* on both pancreas and kidney development.

GCK (MODY2) is the glucose sensor in the beta cell, and several inactivating mutations in *GCK* lead to impaired enzymatic activity and hyperglycemia (9). However, as GCK also processes glucose in hepatocytes, the individual impacts of the gene mutation on the two tissues and their synergistic role in the overall phenotype in MODY2 patients are not fully understood. CEL (MODY8), expressed in acinar cells, is a carboxyl ester lipase (10), and patients with *CEL* mutations develop pancreatic exocrine disease preceding diabetes. It is not known whether exocrine pancreatic dysfunction directly contributes to the diabetes phenotype in these patients. Thus, in both these scenarios, the differentiation of the respective MODY-hiPSCs along the pancreatic (endocrine and exocrine) lineage would potentially unmask mechanisms by which *GCK* and *CEL* mutations underlie defects in beta cell function and/or promote diabetes in humans.

In all the studies discussed above, the MODY-hiPSCs present a timely opportunity to pursue preclinical proof-of-concept experiments. Correcting the mutations using transcription activator-like effector nucleases (11) would allow for the comparison of the pancreatic differentiation potential of these repaired MODY-hiPSCs with their autologous mutant counterparts. Subsequent studies utilizing the repaired isogenic controls and resulting in the reversion of *in vitro* derived phenotype would be strong proof of successful disease modeling. This approach is particularly appealing for MODY given the known genetic variants of each form.

In summary, we report the successful derivation of MODY-hiPSCs as a powerful resource for studying the role of these MODY genes/transcription factors in the development of human pancreas and beta cells and in the regulation of beta cell secretory function.

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